Evaluation Report
proficiency test

DLA ptAUS4 (2020)

Fish Species-Screening :

Black halibut *(Reinhardtius hippoglossoides)*, Atlantic salmon *(Salmo salar)*, Saithe *(Pollachius virens)* and one species of trout

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Allgemeine Informationen zur Eignungsprüfung (EP)
General Information on the proficiency test (PT)

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EP-Koordinator *PT-Coordinator*	Alexandra Scharf MSc.
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (7. Januar 2021) Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 7. Januar 2021
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung nachstehende Leistungen im Unterauftrag vergeben: Proteinbestimmung As part of the present proficency test the following services were subcontracted: protein determination
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

Contents

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

2. Realisation

2.1 Test material

Four different PT samples with possible contents of freeze-dried animal foods from Black halibut (*Reinhardtius hippoglossoides*), Atlantic salmon (*Salmo salar*), Saithe (Pollachius virens), and a trout species were provided for qualitative determination. The parameters were mixtures with maltodextrin with contents of 25-39%.

The raw materials for the fish species used were commercial fish products (whole fish). The fish were stored at -20°C. They were then manually minced and lyophilized at -50°C for 72 hours. The water content loss was supplemented to 100% by adding maltodextrin according to the previously determined wet weights (see Table 1). These mixtures were ground and then sieved (mesh 800 µm). The corresponding fish species in samples 1-4 are shown in Table 2.

After homogenization, the samples were filled into portions of approximately 25 g in metallized PET film bags.

Table 1: Composition of the DLA samples.

Ingredients	**Samples 1 - 4**
Maltodextrin	61 - 75 %
Fish contents (dry weight)	25 - 39 %

Tabelle 2: Fish species in samples 1-4.

Ingredients	Sample 1	Sample 2	Sample 3	Sample 4
Atlantic salmon *(Salmo solar) (protein 22,5%)*	positive	negative	negative	negative
Black halibut *(Rheinhardtius hipo-glossoides) (protein 14%)*	negative	positive	negative	negative
Saithe *(Pollachius virens) (protein 22,2%)*	negative	negative	positive	negative
Rainbow trout *(Oncorhynchus mykiss)*** *(protein 20,2%)*	negative	negative	negative	positive

* Protein contents of the EP samples (including maltodextrin) according to laboratory analysis (total nitrogen according to Kjeldahl with general factor F=6.25).
** The declared trout *(Salmo trutta)* was identified as rainbow trout *(Oncorhynchus mykiss)*.

Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

2.1.1 Homogeneity

The **mixture homogeneity before bottling** of samples 2-4 was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 2-4 showed a probability of 80%, 89% or 100%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave a HorRat value 1,2, 1,0 or 1,8. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].
The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the PT samples was approx. 0,25 - 0,31 (21,1°C-22,2°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of the test materials (sample 1 to 4) were sent to every participating laboratory in the 22th week of 2020. The testing method was optional. The tests should be finished at August 7th 2020 the latest.

With the cover letter along with the sample shipment the following information was given to participants:

There are 4 different samples each containing one of the following fish species: ***Black halibut (Reinhardtius hippoglossoides), Atlantic salmon (Salmo salar), Saithe (Pollachius virens) or Trout (Salmo trutta).***
The parameters are present in the matrix ***Fish Product*** *(freeze dried).*
The evaluation of results is ***strictly qualitative (positive / negative).***

Note: *Samples should be stored refrigerated (2-10 °C) upon arrival.*

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. The results given as positive/negative were evaluated.
Queried and documented were the indicated results and details of the test methods like specificities, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

7 of 8 participants submitted at least one result in time. One participant did not submit any results.

3. Evaluation

Different protein-based methods (e.g. isoelectric focusing, ELISA) and DNA-based methods for the determination of fish species in foods are eventually using different pH-gradients, antibodies and target-DNA, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different valuation of the presence and/or content of the analyte. Furthermore, matrix and/or processing can strongly influence the detectability of fish species, especially when protein-based methods are used [19].

3.1 Agreement with consensus values from participants

The qualitative evaluation of the protein and DNA-based results of each participant was based on the agreement of the indicated results (positive or negative) with the **consensus values from participants**. A consensus value is determined if ≥ 75% positive or negative results are available for a parameter.
The assessment will be in the form that the number of matching results followed by the number of samples for which a consensus value was obtained is indicated. Behind that the agreement is expressed as the percentage in parentheses.

3.2 Agreement with spiking of samples

The qualitative evaluation of the protein and DNA-based results of each participant was based on the agreement of the indicated results (positive or negative) with the **spiking of the four PT-samples**.
The assessment will be in the form that the number of matching results followed by the number of samples is indicated. Behind that the agreement is expressed as the percentage in parentheses.

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

No protein-based results were submitted, therefore only qualitative evaluation for each parameter was performed for DNA-based methods, such as PCR and sequencing.

The participant results and evaluation are tabulated as follows:

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive				
Number negative				
Percent positive				
Percent negative				
Consensus value				
Spiking				

4.1 Proficiency Test Atlantic salmon (*Salmo solar*)

Qualitative valuation of the DNA-based results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
3	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
7	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
5	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	BDT	
2	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFF-ID	
4	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SGS	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	6	0	0	0
Number negative	0	6	6	6
Percent positive	100	0	0	0
Percent negative	0	100	100	100
Consensus value	positive	negative	negative	negative
Spiking	positive	negative	negative	negative

Methods:

ASU = ASU §64 Methode/method

BDT = BigDyeTerminator V1.1, AppliedBiosystems/ ThermoFisher

SFF-ID = Sure Food Fish ID, R-Biopharm / Congen

SGS = SGS All Species ID DNA Analyser, ThermoFisher

Comments:

The consensus values of results are in qualitative agreement with the spiking of the samples.

4.2 Proficiency Test Trout (*Salmo trutta*)

Qualitative valuation of the DNA-based results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	negative	negative	negative	4/4 (100%)	4/4 (100%)	ASU	Sample 4 w as evaluated by DLA as negative because it w as identified as rainbow trout
3	negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	ASU	
7	negative	negative	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
5	negative	negative	negative	negative	4/4 (100%)	4/4 (100%)	BDT	
2	negative	positive	negative	negative	3/4 (75%)	3/4 (75%)	SFF-ID	
6	negative	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFF-ID	
4	negative	negative	negative	negative	4/4 (100%)	4/4 (100%)	SGS	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	1	0	1
Number negative	7	6	7	6
Percent positive	0	14	0	14
Percent negative	100	86	100	86
Consensus value	negative	negative	negative	negative
Spiking	negative	negative	negative	negative

Methods:

ASU = ASU §64 Methode/method

BDT = BigDyeTerminator V1.1, AppliedBiosystems/ ThermoFisher

SFF-ID = Sure Food Fish ID, R-Biopharm / Congen

SGS = SGS All Species ID DNA Analyser, ThermoFisher

Comments:

The consensus values of results are in qualitative agreement with the spiking of the samples.

Salmo trutta has not been added to any of the samples. The fish species in sample 4 was identified as rainbow trout (Oncorhynchus mykiss). One participant each reported a positive result for Salmo trutta in sample 2 and 4, respectively.

4.3 Proficiency Test Rainbow trout (*Oncorhynchus mykiss*)

Qualitative valuation of the DNA-based results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
7	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	Sample 4 was evaluated as positive by DLA, as identified as rainbow trout.
5	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	BDT	
4	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SGS	Sample 4 was evaluated as positive by DLA, as identified as rainbow trout.

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	0	4
Number negative	4	4	4	0
Percent positive	0	0	0	100
Percent negative	100	100	100	0
Consensus value	negative	negative	negative	positive
Spiking	negative	negative	negative	positive

Methods:

ASU = ASU §64 Methode/method

BDT = BigDyeTerminator V1.1, AppliedBiosystems/ ThermoFisher

SGS = SGS All Species ID DNA Analyser, ThermoFisher

Comments:

The consensus values of results are in qualitative agreement with the experimental identification of rainbow trout in sample 4.

4.4 Proficiency Test Black halibut (*Reinhardtius hippoglossoides*)

Qualitative valuation of the DNA-based results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
3	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
7	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
5	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	BDT	
2	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFF-ID	
4	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SGS	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	6	0	0
Number negative	6	0	6	6
Percent positive	0	100	0	0
Percent negative	100	0	100	100
Consensus value	negative	positive	negative	negative
Spiking	negative	positive	negative	negative

Methods:

ASU = ASU §64 Methode/method

BDT = BigDyeTerminator V1.1, AppliedBiosystems/ ThermoFisher

SFF-ID = Sure Food Fish ID, R-Biopharm / Congen

SGS = SGS All Species ID DNA Analyser, ThermoFisher

Comments:

The consensus values of results are in qualitative agreement with the spiking of the samples.

4.5 Proficiency Test Saithe (*Pollachius virens*)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
3	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
7	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
5	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	BDT	
2	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFF-ID	
6	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFF-ID	
4	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SGS	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	7	0
Number negative	7	7	0	7
Percent positive	0	0	100	0
Percent negative	100	100	0	100
Consensus value	negative	negative	positive	negative
Spiking	negative	negative	positive	negative

Methods:

ASU = ASU §64 Methode/method

BDT = BigDyeTerminator V1.1, AppliedBiosystems/ ThermoFisher

SFF-ID = Sure Food Fish ID, R-Biopharm / Congen

SGS = SGS All Species ID DNA Analyser, ThermoFisher

Comments:

The consensus values of results are in qualitative agreement with the spiking of the samples.

5. Documentation

5.1 Details by the participants

Note: Information given in German was translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1.1 DNA-based Methods: Atlantic salmon (Salmo salar)

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
ASU	1	19.06.20	positive	negative	negative	negative	0,001	DNA	
ASU	3	15.06.20	positive	negative	negative	negative	5	DNA	o.A.
ASU	7	16.06.20	positive	negative	negative	negative			Cytb PCR with consecutive sequencing according to §64
BDT	5		positive	negative	negative	negative			BigDyeTerminator Cycle Sequencing Kit V1.1; Fa. AppliedBiosystems /ThermoFisher
SFF-ID	2		positive	negative	negative	negative	<1	food	SureFood® Fish ID Salmo salar IAAC
SGS	4		positive	negative	negative	negative	10 - 60 pg	DNA in 60 - 43320 pg background-DNA	SGS All Species ID Fish DNA Analyser - Next Generation Sequencing on Ion Torrent platform

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
ASU	1	ASU L10.00-12			
ASU	3	L 10.00-12:2012-08	Cytochrome b	CTAB, Proteinase K, Chloroform; DNeasy Mericon Food Kit; RT-PCR (QuantStudio/ABI): 45 cycles	
ASU	7		Cytb	DNA extraction using Wizard, Cytb PCR 50 cycles, gel electrophoresis, elution using ReliaPrep from Promega, sequencing, sequence comparison in GenBank with BLAST	
BDT	5	§64 LFGB, L10.00-12; CEN/TS 17303: 2019 (2019-03)	Cytochrome B; COI	Extraction: DNeasy Mericon Food; Qiagen/PCR: GoTaq G2 Flexi DNA Polymerase; Promega (CytB), HotStar Taq DNA Polymerase; Qiagen (COI); 40 cycles/ microchip electrophoresis/ QIAQuick PCR Purification Kit, Fa. Qiagen/ sequencing PCR BigDye Terminator Cycle Sequencing Kit V1.1, Fa. AB/ThermoF./DyeEx Spin 2.0 Kit	Sequencing by ABI310; Percentage agreement 100% (CytB); 99-100% (COI).
SFF-ID	2	S6306	Salmo salar	SureFood® Prep Basic	K00
SGS	4				

5.1.2 DNA-based Methods: Trout (Salmo trutta)/ Rainbow trout (Oncorhynchus mykiss)

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
ASU	1	19.06.20	negative	negative	negative	positive	0,001	DNA	
ASU	3	15.06.20	negative	negative	negative	positive	5	DNA	o.A.
ASU	7	16.06.20	negative	negative	negative	negative			Cytb PCR with consecutive sequencing according to §64
BDT	5		negative	negative	negative	negative (Trout)			BigDyeTerminator Cycle Sequencing Kit V1.1; Fa. AppliedBiosystems/ThermoFisher
BDT	5		negative	negative	negative	positive (Rainbow trout)			BigDyeTerminator Cycle Sequencing Kit V1.1; Fa. AppliedBiosystems/ThermoFisher
SFF-ID	2		negative	positive	negative	negative	<0,1	food	SureFood® Fish ID Salmo trutta IAAC
SFF-ID	6	03.08.20	negative	negative	negative	negative	0,01	DNA	Congen SureFood Fish ID
SGS	4		negative	negative	negative	negative	10 - 60 pg	DNA in 60 - 43320 pg background-DNA	SGS All Species ID Fish DNA Analyser - Next Generation Sequencing on Ion Torrent platform

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
ASU	1	ASU L10.00-12			Rainbow trout (Oncorhynchus mykiss)
ASU	3	L 10.00-12:2012-10	Cytochrome b	CTAB, Proteinase K, Chloroform; DNeasy Mericon Food Kit; RT-PCR (QuantStudio/ABI): 45 cycles	
ASU	7		Cytb	DNA extraction using Wizard, Cytb PCR 50 cycles, gel electrophoresis, elution using ReliaPrep from Promega, sequencing, sequence comparison in GenBank with BLAST	According to sequencing, sample 4 is Oncorhynchus mykiss.
BDT	5	§64 LFGB, L10.00-12; CEN/TS 17303: 2019 (2019-03)	Cytochrome B; COI	Extraction: DNeasy Mericon Food; Qiagen/PCR: GoTaq G2 Flexi DNA Polymerase; Promega (CytB), HotStar Taq DNA Polymerase; Qiagen (COI); 40 cycles/microchip electrophoresis/QIAQuick PCR Purification Kit, Fa. Qiagen/Sequencing PCR BigDye Terminator Cycle Sequencing Kit V1.1, Fa. AB/ThermoF./DyeEx Spin 2.0 Kit	Trout (Salmo trutta); sequencing by ABI310; percentage agreement 90% (CytB); 88% (COI).
BDT	5	§64 LFGB, L10.00-12; CEN/TS 17303: 2019 (2019-03)	Cytochrome B; COI	Extraction: DNeasy Mericon Food; Qiagen/PCR: GoTaq G2 Flexi DNA Polymerase; Promega (CytB), HotStar Taq DNA Polymerase; Qiagen (COI); 40 cycles/microchip electrophoresis/QIAQuick PCR Purification Kit, Fa. Qiagen/Sequencing PCR BigDye Terminator Cycle Sequencing Kit V1.1, Fa. AB/ThermoF./DyeEx Spin 2.0 Kit	Rainbow trout (Oncorhynchus mykiss) sequencing using ABI310; percentage agreement 99-100%.
SFF-ID	2	S6305	Salmo trutta	SureFood® Prep Basic	K00
SFF-ID	6	as per kit instructions	as per kit instructions	as per kit instructions	
SGS	4				Instead of Salmo trutta, Oncorhynchus mykiss was identified (sample 4).

5.1.3 DNA-based Methods: Black halibut (Reinhardtius hippoglossoides)

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
ASU	1	19.06.20	negative	positive	negative	negative	0,001	DNA	
ASU	3	15.06.20	negative	positive	negative	negative	5	DNA	o.A.
ASU	7	16.06.20	negative	positive	negative	negative			Cytb PCR with consecutive sequencing according to §64
BDT	5		negative	positive	negative	negative			BigDyeTerminator Cycle Sequencing Kit V1.1; Fa. AppliedBiosystems/ThermoFisher
SFF-ID	2		negative	positive	negative	negative	<1	food	SureFood® Fish ID 3plex Halibut IAAC
SGS	4		negative	positive	negative	negative	10 - 60 pg	DNA in 60 - 43320 pg background-DNA	SGS All Species ID Fish DNA Analyser - Next Generation Sequencing on Ion Torrent platform

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
ASU	1	ASU L10.00-12		DNA extraction MN spin-food kit, PCR, ident via sequencing	
ASU	3	L 10.00-12:2012-07	Cytochrome b	CTAB, Proteinase K, Chloroform; DNeasy Mericon Food Kit; RT-PCR (QuantStudio/ABI): 45 cycles	
ASU	7		Cytb	DNA extraction using Wizard, Cytb PCR 50 cycles, gel electrophoresis, elution using Relia-Prep from Promega, sequencing, sequence comparisons with GenBank using BLAST	
BDT	5	§64 LFGB, L10.00-12; CEN/TS 17303: 2019 (2019-03)	Cytochrome B; COI	Extraction: DNeasy Mericon Food; Qiagen/PCR: GoTaq G2 Flexi DNA Polymerase; Promega (CytB), HotStar Taq DNA Polymerase; Qiagen (COI); 40 cycles/microchip electrophoresis/QIAQuick PCR Purification Kit, Fa. Qiagen/Sequencing PCR BigDye Terminator Cycle Sequencing Kit V1.1, Fa. AB/ThermoF./DyeEx Spin 2.0 Kit	Sequencing by ABI310; Percentage agreement 99-100%.
SFF-ID	2	S6201	Reinhardtius hippoglossoides	SureFood® Prep Basic	K00
SGS	4			Extraction Kit: Macherey-Nagel Nucleo Spin Food/Quantification Qubit/PCR All Species ID/Gel Electrophoresis/Purification with Agencourt/Ion Chef/Ion S5 Sequencer	

5.1.4 DNA-based Methods: Saithe (Pollachius virens)

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
ASU	1	19.06.20	negative	negative	positive	negative	0,001	DNA	
ASU	3	15.06.20	negative	negative	positive	negative	5	DNA	o.A.
ASU	7	16.06.20	negative	negative	positive	negative			Cytb PCR with consecutive sequencing according to §64
BDT	5		negative	negative	positive	negative			BigDyeTerminator Cycle Sequencing Kit V1.1; Fa. AppliedBiosystems/ThermoFisher
SFF-ID	2		negative	negative	positive	negative	<1		SureFood® Fish ID Pollachius virens IAAC
SFF-ID	6	03.08.20	negative	negative	positive	negative	0,01	DNA	Congen SureFood Fish ID
SGS	4		negative	negative	positive	negative	10 - 60 pg	DNA in 60 - 43320 pg background-DNA	SGS All Species ID Fish DNA Analyser - Next Generation Sequencing on Ion Torrent platform

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
ASU	1	ASU L10.00-12			
ASU	3	L 10.00-12:2012-09	Cytochrome b	CTAB, Proteinase K, Chloroform; DNeasy Mericon Food Kit; RT-PCR (QuantStudio/ABI): 45 cycles	
ASU	7		Cytb	DNA extraction using Wizard, Cytb PCR 50 cycles, gel electrophoresis, elution using ReliaPrep from Promega, sequencing, sequence comparison with BLAST using GenBank.	
BDT	5	§64 LFGB, L10.00-12; CEN/TS 17303: 2019 (2019-03)	Cytochrome B; COI	Extraction: DNeasy Mericon Food; Qiagen/PCR: GoTaq G2 Flexi DNA Polymerase; Promega (CytB), HotStar Taq DNA Polymerase; Qiagen (COI); 40 cycles/microchip electrophoresis/QIAQuick PCR Purification Kit, Fa. Qiagen/Sequencing PCR BigDye Terminator Cycle Sequencing Kit V1.1, Fa. AB/ThermoF./DyeEx Spin 2.0 Kit	Sequencing by ABI310; Percentage agreement 99-100%.
SFF-ID	2	S6309	Pollachius virens	SureFood® Prep Basic	K00
SFF-ID	6	as per kit instructions	as per kit instructions	as per kit instructions	
SGS	4				

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA -ptAUS4 Sample 2

Weight whole sample	0,34	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	48,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	2,52	46	36,5
2	2,51	54	43,0
3	2,51	50	39,8
4	2,51	46	36,7
5	2,53	39	30,8
6	2,49	50	40,2
7	2,49	47	37,8
8	2,48	54	43,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	48,3	Particles
Standard deviation	5,13	Particles
χ^2 (CHI-Quadrat)	3,81	
Probability	**80**	%
Recovery rate	80	%

Normal distribution		
Number of samples	8	
Mean	38,5	mg/kg
Standard deviation	4,09	mg/kg
rel. Standard deviaton	10,6	%
Horwitz standard deviation	9,2	%
HorRat-value	**1,2**	
Recovery rate	80	%

Microtracer Homogeneity Test
DLA -ptAUS4 Sample 3

Weight whole sample	0,40	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	43,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	2,52	56	44,4
2	2,49	47	37,8
3	2,49	55	44,2
4	2,50	57	45,6
5	2,52	62	49,2
6	2,53	48	37,9
7	2,52	54	42,9
8	2,49	55	44,2

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	54,2	Particles
Standard deviation	4,80	Particles
χ^2 (CHI-Quadrat)	2,98	
Probability	**89**	%
Recovery rate	99	%

Normal distribution		
Number of samples	8	
Mean	43,3	mg/kg
Standard deviation	3,83	mg/kg
rel. Standard deviaton	8,9	%
Horwitz standard deviation	9,1	%
HorRat-value	**1,0**	
Recovery rate	99	%

Microtracer Homogeneity Test
DLA -ptAUS4 Sample 4

Weight whole sample	0,34	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	40,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	2,53	40	31,6
2	2,51	31	24,7
3	2,48	27	21,8
4	2,51	27	21,5
5	2,51	39	31,1
6	2,54	38	29,9
7	2,52	25	19,8
8	2,50	32	25,6

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	32,4	Particles
Standard deviation	5,82	Particles
χ^2 (CHI-Quadrat)	7,32	
Probability	**40**	%
Recovery rate	64	%

Normal distribution		
Number of samples	8	
Mean	25,8	mg/kg
Standard deviation	4,63	mg/kg
rel. Standard deviaton	18,0	%
Horwitz standard deviation	9,8	%
HorRat-value	**1,8**	
Recovery rate	64	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter:

PT number	***DLA ptAUS4 (2020)***
PT name	***Fish-Screening - 4 Samples qualitative: Black halibut (Reinhardtius hippoglossoides), Atlantic salmon (Salmo salar), Saithe (Pollachius virens) and Trout (Salmo trutta) in Fish Product (freeze-dried, one species per sample)***
Sample matrix	*Samples 1-4:* *Fish powder / ingredients: Fish freeze-dried, maltodextrin* *(amount of fish corresponds to 100% fresh fish)*
Number of samples and sample amount	*4 different Samples 1-4: 25 g each*
Storage	*Samples 1-4: room temperature (long term cooled 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative:**Black halibut (Reinhardtius hippoglossoides), Atlantic salmon (Salmo salar), saithe (Pollachius virens) and trout (Salmo trutta)*** *Samples 1-4: one species per sample*
Methods of analysis	*The analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*
Result sheet	*One result each should be determined for Samples 1-4.* *The results should be filled in the result submission file.*
Units	*posititv / negativ (limit of detection %)*
Number of digits	*at least 2*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Last Deadline	***the latest August 07th 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Alexandra Scharf M.Sc.*

** Control of mixture homogeneity and qualitative testings are carried out by DLA. Any testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.*

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]

[The address data of the participants were deleted for publication of the evaluation report.]

7. Index of references

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